"ANTIDIABETIC AND ANTIOXIDANT EFFECTS OF ETHANOLIC EXTRACT OF *Ficus carica* LEAVES IN ALLOXAN INDUCED DIABETIC RAT"

Dissertation submitted to

THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSITY

CHENNAI

In partial fulfillment of the requirements for the award of degree of

MASTER OF PHARMACY

in

PHARMACOLOGY

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April-2014

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Dedicated to my

Parents, Teachers

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I hereby declare that the thesis titled **"ANTIDIABETIC AND ANTIOXIDANT EFFECTS OF ETHANOLIC EXTRACT OF *Ficus carica* LEAVES IN ALLOXAN INDUCED DIABETIC RAT"**submitted in partial fulfillment for the award of degree Master of Pharmacy to The TamilnaduDr. M.G.R. Medical University and carried out at Mohamed Sathak A.J.College of Pharmacy, Chennai, is my original and independent work done under the direct supervision and guidance **Mrs.MALINI SEN, M. Pharm, Associate Professor, Department of Pharmacology** during the academic year 2013-2014 and this thesis contains no material which has been accepted for the award of any degree or diploma of other Universities.

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ACKNOWLEDGEMENT

I wish to express my gratitude towards “GOD – Almighty”, who gave me the strength and courage to fulfill my dream and has showered upon me his choicest blessings.

I take this opportunity to express my heartfelt thanks to all those, who knowingly or unknowingly contributed to the success of my dissertation work.

My heartfelt thanks to my parents and my brother and sisters for their love affection and constantly encouraging, guiding when I thought nothing is happening.

I wishtoexpressmy deepestgratitudeto **Management of Mohamed Sathak Trust**, Chennai and **Management of Mohamed Sathak A.J. College of Pharmacy,** in acknowledging all facilities provided to use at the institution enabling us to do work of this magnitude.

I express my sincere thanks to **Dr. R. Sundararajan,** M.Pharm., Ph.D., Principal, Mohamed Sathak A.J. College of Pharmacy, for his moral encouragement and providing necessary facilities required for my dissertation work.

It is indeed a great pleasure to express my deep sense of gratitude and humble thanks to my guide **Mrs.MALINI SEN,** M. Pharm, Assistant Professor **Mr. J. Gunasekaran,** M. Pharm, (Ph.D.), Associate Professor & **Prof. M. Jagadeesan,** M. Pharm, Head of the Department of Pharmacology, Mohamed Sathak A.J. College Of Pharmacy, Chennai, for his invaluable guidance and constant encouragement that formed the foundation of this project. His discipline, principle, simplicity, the profound knowledge and the subject understanding influenced me a lot. I am proud to say that it has been a most fruitful and enjoyable experience to work under his untiring and dynamic guidance.

I am deeply indebited to the teaching staff especially **Dr. Deepa Sankar,** M. Pharm., Ph.D., Vice Principal, Mohamed Sathak A.J. College of Pharmacy, **M. Komala,** M. Pharm, (Ph.D), HOD and **Mrs. N. B. SanthaSheela,** M. Pharm.,(Ph.D)., Associate Professor, Department of Pharmaceutics and other teaching staff including **Mr. S. Ramachandran, and Mr. Shakti Saravanan,** M. Pharm.,who was always a source of knowledge and inspiration to me and also for their prompt assistance and cooperative attitude.

I thank **Mr. A. Mohamad Jamaludeen,** lab assistant, Department of Pharmacology for his timely help.

I wish to express my special thanks to librarians **Dr. M. Amudha, M.A.L.I.Sc., Ph.D.,** and **Mrs. Kumari, M.A.L.I.Sc.,** for helping me in collecting my reference material.

I also wish to express my sincere thanks to **Mr. Arunachalam and Mr. Mani**, for his technical support and advices given during the entire course of my project work. With his dynamic approach he boosted my morale, which helped me in completion of this dissertation.

Friends are integral part of life, so I take this opportunity to thank my dearest friends **Aleemsha, Mohamed Faisal, Padmapriya, Neelima Maddali, Ananthula Prudhvidhar, Anandraj Kumar, Dinesh Kumar, Ramdoss, and Saravanan** who always pushed my confidence and creativity to the eventual extent of my mind and for their unflinching support and co-operation during my dissertation.

Also I want to thank all teaching and non teaching staff, who directly or indirectly helped me in completing this dissertation work successfully.

JAYAKUMAR SP

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ABBREVATIONS

µg	-	Microgram
AL	-	Anjir Leaves
ALB	-	Albumin
ANOVAs	-	Analysis of Variance
CAT	-	Catalase
GLO	-	Globulin
gm	-	Gram
I.U	-	International units
LD	-	Lethal Dosage
LPO	-	lipid peroxidase
mg	-	Milligram
mg/dl	-	Milligram per deciliter
mg/kg	-	Milligram per kilogram
SOD	-	Superoxide Dismutase
TP	-	Total Protein
U/L	-	Unit per Liter

1. INTRODUCTION

1.1. Medicinal Plants

The therapeutic properties of medicinal plants are conditioned by the presence of active substances, such as alkaloids, flavonoids, glycosides, vitamins, tannins, and coumarin compounds, which physiologically affect humans and animals or which are biologically active in relation to the causative agents of various diseases. A special group of medicinal plants are antibiotics [1].

Harvested medicinal plants are usually dried in special crop dryers, in lofts, or in the shade. To obtain essential oils and juices from certain medicinal plants only freshly harvested material can be used. Dried medicinal plants are used in pharmaceutical practice, in galenics production, and in the chemical and pharmaceutical industry. The composition and quantity of active substances found in different organs of medicinal plants vary and change in the course of the year as a result of the aging of the plant and habitat conditions [2]. Those parts of the plants in which the largest quantity of these substances accumulates are collected first.

Many medicinal plants are no longer used, owing to the availability of more effective drugs. However, more than 30 percent of all drugs obtained in the USSR are of plant origin; for the most part they are less toxic than synthetic agents and have no side effects. However, treatment with medicinal plants must be conducted under the supervision of a physician. More than 30,000 tons of raw materials from approximately 220 species of medicinal plants are used annually in the USSR. Of the plants collected, more than 75 percent of the species grow wild, accounting for 50 percent of the total weight. The rest are cultivated on 23 sovkhozes of the Ministry of Medicinal Industry. The opium poppy and peppermint are also cultivated on kolkhozes. A number of medicinal plants are cultivated and gathered in their natural habitats, including marshmallow, henbane, valerian, ginseng, Saint-John’s-wort, plantain, motherwort, and burmarigold. In gathering the raw materials, only part of the plant should be dug up or cut off in order to ensure its

self-renewal. Only a few medicinal plants are imported into the USSR. A few dozen species of medicinal plants are exported annually from the USSR, including several thousand tons of licorice roots. Many medicinal plants are used in the food industry, in the perfume industry and in metallurgy [3].

The All-Union Scientific Research Institute of Medicinal Plants, a number of institutes of the Academy of Sciences of the USSR and of the Academies of Sciences of the Soviet republics, pharmaceutical institutes (pharmaceutical departments), botanical gardens, and other scientific research and educational institutions are searching for new preparations of plant origin, cultivating medicinal plants and studying their natural properties, and creating a rational regime for their use [3].

1.2. Diabetes

Diabetes is a metabolic disorder that is characterized by high blood glucose and either insufficient or ineffective insulin. 5.9% of the population in the United States has diabetes, and diabetes is the seventh leading cause of death in India. Diabetes is a chronic disease without a cure, however, with proper management and treatment, diabetics can live a normal, healthy lives. There are two main types of diabetes, Type I and Type II, described below [4].

Type I Diabetes

Insulin-dependant is caused by damage to the pancreas. The pancreas contains beta cells, which make insulin. With Type I diabetes, the deficiency of insulin is due to a decline in the number of beta cells the pancreas contains. It appears that certain genes make Type I diabetics more susceptible, but a triggering factor (usually a viral infection) sets it off. In most people with Type I diabetes, the immune system makes a mistake, attacking the beta cells and causing them to die. Without the beta cells, you cannot produce insulin. Glucose then builds up in the blood and causes diabetes [5]. Type I diabetes exhibits the following warning signs.

1. Losing weight without trying
2. An increased need to urinate
3. Increased hunger
4. Increased thirst
5. Trouble seeing
6. Feeling tired and/or
7. Going into a coma

For Type I diabetes, treatment usually consists of a healthy diet, exercise, and insulin shots to replace the insulin that your body no longer produces. Most insulin-dependent diabetics test their blood at least four times per day to monitor their blood's glucose level. This is necessary to keep their blood glucose within certain limits. If blood glucose is not monitored, and if insulin levels are not kept in check, three things may happen:

Ketoacidosis – occurs when your blood glucose levels are highly elevated, by either eating too much or taking too little insulin, by stress or illness. In this case, there is too little insulin in the blood. Your body then begins breaking down fat for energy, producing chemicals called ketones. Ketones can make you throw up, have difficulty breathing, cause excessive thirst, cause dry, itchy skin, or even cause coma.

Hypoglycemia – occurs when blood glucose levels become too low. It can be by taking too much insulin, eating too little, skipping meals, eating at the wrong time, exercising too intensely or for too long, or by drinking alcohol on an empty stomach. If your blood glucose is too low you may feel hungry, confused, tired, shaky or nervous [6].

Complications – elevated glucose levels in the blood over time can hurt your organs. Diabetes can damage kidneys, eyes and nerves, and makes heart and blood

vessel disease more likely. Diabetics can defend themselves from complications by keeping their glucose levels under control [7].

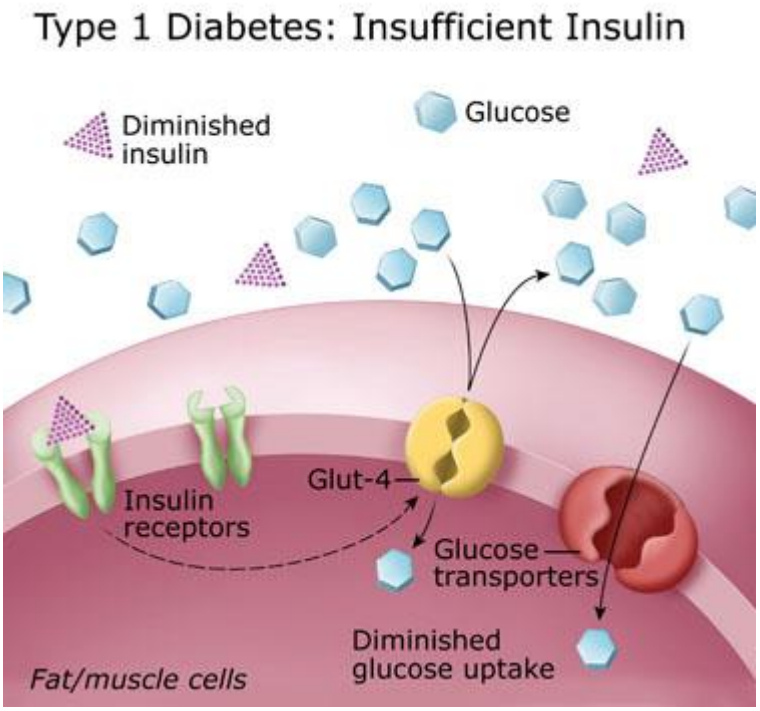

Figure 1: Type 1 Diabetes: Insufficient Insulin

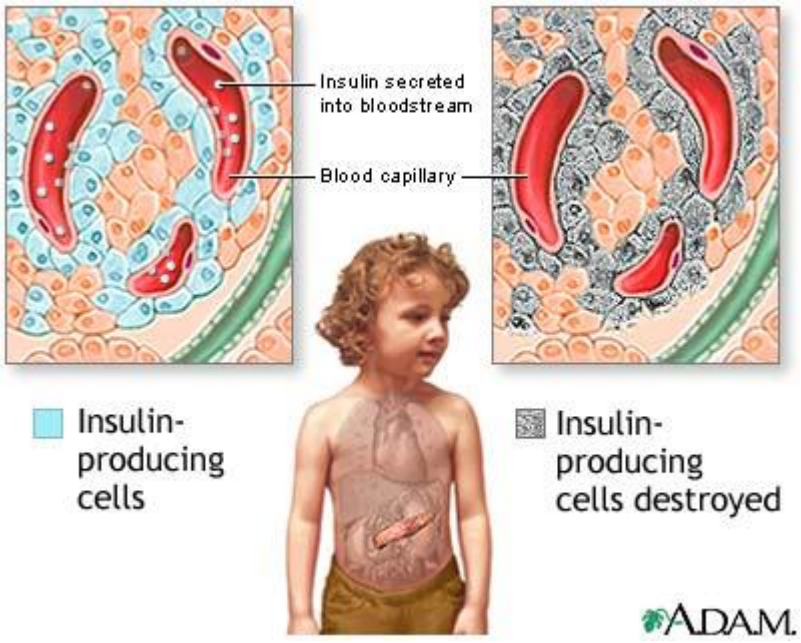

Figure 2: Information on Type 1 Diabetes

Type II Diabetes

Type II diabetes is the most common form of diabetes, with about 90% of diabetes falling into the Type II category. With Type II diabetes, glucose builds up in the blood – not because not enough insulin is present, but probably because cells lose their insulin receptors and become less sensitive to insulin. Type II diabetes usually (though not always) occurs in individuals who are over 40 years of age who are overweight.

Type II diabetes produces mild symptoms, and can be controlled with a healthy diet, exercise and weight loss. Type II diabetics should also monitor their glucose levels to be sure they are maintaining healthy levels. In some cases, weight loss, diet and exercise are not enough to control the glucose levels. In those cases, your physician may control your diabetes by prescribing diabetes pills or insulin shots. Type II diabetes can cause three types of problems.

1. High Blood Sugar – high glucose levels in the blood are most likely when you're sick or under a lot of stress. High blood sugar can cause you to have a headache, blurry vision, excessive thirst and an increased need to urinate, and cause dry, itchy skin. Though less of a problem with Type II diabetes, ketones can build up in the blood when Type II diabetics have symptoms of high blood sugar, or when they are sick.

2. Low Blood Sugar – When blood sugar falls to low you may feel tired, shaky, nervous, hungry or confused. It may be caused by taking too much diabetes medicine, eating too little or skipping meals, exercising too intensely or for too long, or from drinking alcohol without eating.

3. Complications – Elevated blood glucose over many years can hurt organs, including the eyes, kidneys, and eyes. It can also make heart and blood vessel disease more likely. The best defense against complications is a careful monitoring of blood glucose, a healthy diet and exercise [8].

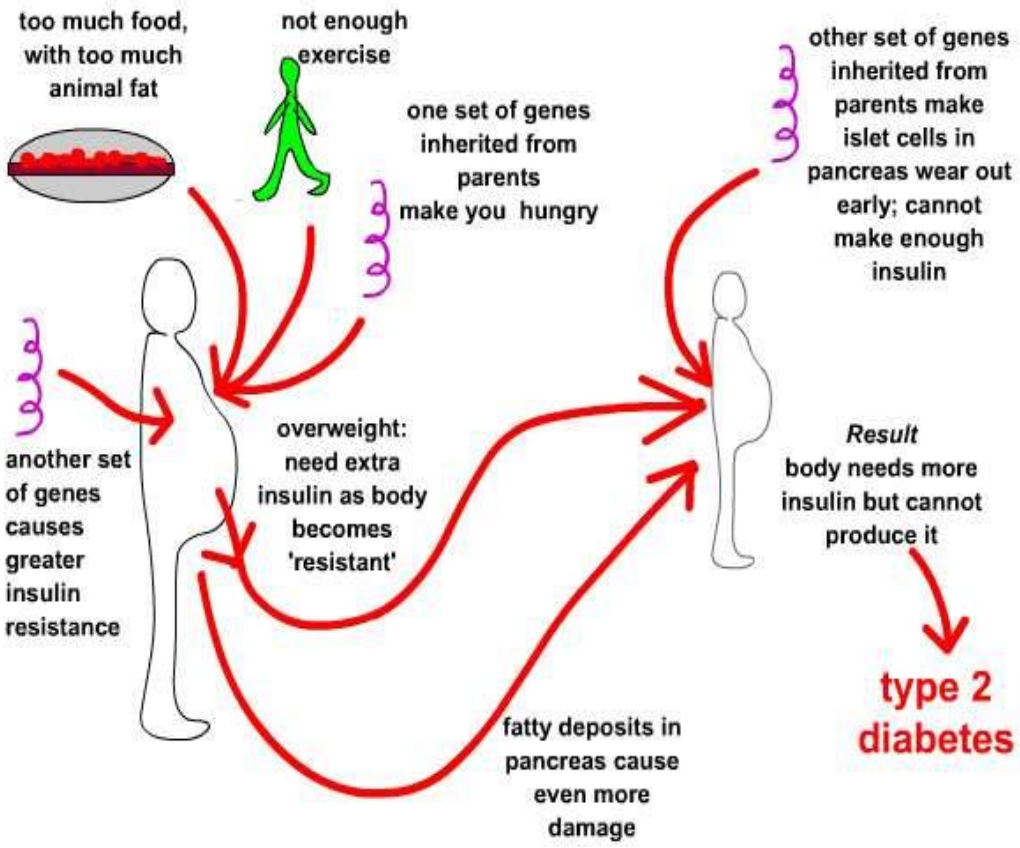

Figure 3: Factors Contributing To Type 2 Diabetes

Other Forms of Diabetes

Gestational diabetes occurs during pregnancy and affects about 4% of all pregnant women, approximately 135,000 cases in the U.S. per year.

Gestational diabetes usually goes away after pregnancy, but once you've had gestational diabetes, your chances are higher that it will happen in future pregnancies. In some women pregnancy uncovers Type 1 or Type 2 diabetes and these women will need to continue diabetes treatment after pregnancy.

There seems to be a link between the tendency to have gestational diabetes and Type 2 diabetes, and many women who had gestational diabetes develop Type 2 diabetes later on. Gestational diabetes and Type 2 diabetes both involve insulin resistance. Certain basic lifestyle changes may help prevent diabetes after gestational diabetes.

Pre-diabetes is a condition that causes a person's blood sugar levels to be higher than normal but not high enough to be diagnosed with diabetes. The American Diabetes Association estimates that there are 41 million Americans that have pre-diabetes in addition to the 18.2 million with diabetes.

Before people develop Type 2 diabetes, they almost always have "pre-diabetes." Recent research has shown that some long-term damage to the body, especially the heart and circulatory system, may start during pre-diabetes.

Risks for Diabetes

1. Individuals with parents or siblings with diabetes
2. People over the age of 45
3. People who are overweight
4. People who do not exercise regularly
5. People with low HDL cholesterol or high triglycerides
6. Certain racial and ethnic groups (African Americans, Latinos, Asians and Native Americans)
7. Women who had gestational diabetes or who had a baby weighing 9 pounds or more at birth.

Antioxidant

An antioxidant is a substance that when present in low concentrations relative to the oxidizable substrate significantly delays or reduces oxidation of the substrate

Antioxidants get their name because they combat oxidation. They are substances that protect other chemicals of the body from damaging oxidation reactions by reacting with free radicals and other reactive oxygen species within the body, hence hindering the process of oxidation. During this reaction the antioxidant sacrifices itself by becoming oxidized. However, antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical. Therefore, there is a constant need to replenish antioxidant resources, whether endogenously or through supplementation [9].

Free Radical Formation

Atoms are most stable in the ground state. An atom is considered to be "ground" when every electron in the outermost shell has a complimentary electron that spins in the opposite direction. By definition a free radical is any atom with at least one unpaired electron in the outermost shell, and is capable of independent existence [10]. A free radical is easily formed when a covalent bond between entities is broken and one electron remains with each newly formed atom. Free radicals are highly reactive due to the presence of unpaired electron(s). The following literature review addresses only radicals with an oxygen center. Any free radical involving oxygen can be referred to as reactive oxygen species. Oxygen centered free radicals contain two unpaired electrons in the outer shell. When free radicals steal an electron from a surrounding compound or molecule a new free radical is formed in its place. In turn the newly formed radical then looks to return to its ground state by stealing electrons with antiparallel spins from cellular structures or molecules. Thus the chain reaction continues and can be thousands of events long. The electron transport chain (ETC), which is found in the inner mitochondrial membrane, utilizes oxygen to generate energy in the form of adenosine triphosphate (ATP). Oxygen

acts as the terminal electron acceptor within the ETC. The literature suggests that anywhere from 2 to 5% (14) of the total oxygen intake during both rest and exercise have the ability to form the highly damaging superoxide radical via electron escape. During exercise oxygen consumption increases 10 to 20 fold to 35-70 ml/kg/min. In turn, electron escape from the ETC is further enhanced. Thus, when calculated, .6 to 3.5 ml/kg/min of the total oxygen intake during exercise have the ability to form free radicals. Electrons appear to escape from the ETS at the ubiqunone-cytochrome c level [11].

Peroxidation

Polyunsaturated fatty acids (PUFAs) are abundant in cellular membranes and in low-density lipoproteins. The PUFAs allow for fluidity of cellular membranes. A free radical prefers to steal electrons from the lipid membrane of a cell, initiating a free radical attack on the cell known as lipid peroxidation. Reactive oxygen species target the carbon-carbon double bond of polyunsaturated fatty acids. The double bond on the carbon weakens the carbon-hydrogen bond allowing for easy dissociation of the hydrogen by a free radical. A free radical will steal the single electron from the hydrogen associated with the carbon at the double bond. In turn this leaves the carbon with an unpaired electron and hence becomes a free radical. In an effort to stabilize the carbon-centered free radical molecular rearrangement occurs. The newly arranged molecule is called a conjugated diene. The CD then very easily reacts with oxygen to form a proxy radical. The proxy radical steals an electron from another lipid molecule in a process called propagation. This process then continues in a chain reaction [12, 13].

Types of Free Radicals

There are numerous types of free radicals that can be formed within the body. The most common ROS include: the superoxide anion, the hydroxyl radical, singlet oxygen, and hydrogen peroxide Superoxide anions are formed when oxygen acquires an additional electron, leaving the molecule with only one unpaired electron. Within the mitochondria O_2- is continuously being formed. The rate of

formation depends on the amount of oxygen flowing through the mitochondria at any given time. Hydroxyl radicals are short-lived, but the most damaging radicals within the body. These reactions are significant as the substrates are found within the body and could easily interact. Hydrogen peroxide is produced in vivo by many reactions. Hydrogen peroxide is unique in that it can be converted to the highly damaging hydroxyl radical or be catalyzed and excreted harmlessly as water. Glutathione peroxidase is essential for the conversion of glutathione to oxidized glutathione, during which H_2O_2 is converted to water. If H_2O_2 is not converted into water is formed. Singlet oxygen is not a free radical, but can be formed during radical reactions and also cause further reactions. Singlet oxygen violates Hund's rule of electron filling in that it has eight outer electrons existing in pairs leaving one orbital of the same energy level empty. When oxygen is energetically excited one of the electrons can jump to empty orbital creating unpaired electrons [14]. Singlet oxygen can then transfer the energy to a new molecule and act as a catalyst for free radical formation. The molecule can also interact with other molecules leading to the formation of a new free radical.

Physiological Effects

Under normal conditions (at rest) the antioxidant defense system within the body can easily handle free radicals that are produced. During times of increased oxygen flux (i.e. exercise) free radical production may exceed that of removal ultimately resulting in lipid peroxidation. Free radicals have been implicated as playing a role in the etiology of cardiovascular disease, cancer, Alzheimer’s disease, and Parkinson’s disease. While worthy of a discussion these conditions are not the focus of the current literature review. This literature review will only examine the current literature addressing the relationship between free radicals and exercise, which is introduced below. The driving force behind these topics is lipid peroxidation. By preventing or controlling lipid peroxidation the concomitant effects discussed below would be better controlled.

Requirement

Oxygen consumption greatly increases during exercise, which leads to increased free radical production. The body counters the increase in free radical production through the antioxidant defense system. When free radical production exceeds clearance oxidative damage occurs. Free radicals formed during chronic exercise may exceed the protective capacity of the antioxidant defense system, thereby making the body more immune to disease and injury. Therefore, the need for antioxidant supplementation is discussed.

Fatigue

A free radical attack on a membrane usually damages a cell to the point that it must be removed by the immune system. If free radical formation and attack are not controlled within the muscle during exercise a large quantity of muscle could easily be damaged. Damaged muscle could in turn inhibit performance by the induction of fatigue. The role individual antioxidants have in inhibiting this damage has been addressed within the review of the four antioxidants that follows.

Recovery

One of the first steps in recovery from exercise induced muscle damage is an acute inflammatory response at the site of muscle damage. Free radicals are commonly associated with the inflammatory response and are hypothesized to be greatest twenty-four hours after completion of a strenuous exercise session. If this theory were valid then antioxidants would play a major role in helping prevent this damage. However, if antioxidant defense systems are inadequate or not elevated during the post-exercise infiltration period free radicals could further damage muscle beyond that acquired during exercise. This in turn would increase the time needed to recover from an exercise bout [15].

Importance of Free Radicals

This section has focused only on the negatives associated with free radical production. However, free radicals are naturally produced by some systems within

the body and have beneficial effects that cannot be overlooked. The immune system is the main body system that utilizes free radicals. Foreign invaders or damaged tissue is marked with free radicals by the immune system. This allows for determination of which tissue need to be removed from the body. Because of this some question the need for antioxidant supplementation, as they believe supplementation can actually decrease the effectiveness of the immune system.

Antioxidant Defenses

Antioxidant means "against oxidation." Antioxidants work to protect lipids from peroxidation by radicals. Antioxidants are effective because they are willing to give up their own electrons to free radicals. When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken [16]. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system [17].

2. PLANT PROFILE AND REVIEW OF LITERATURE

2.1. Botanical classification

Scientific Name: *Ficus carica*

Other Names: Common Fig, Edible Fig, Anjir, Anjuru, Anjura, Athi, Seemai, Simayathi, Figue (French), Feige (German), Figo (Italian & Portugese)

Taxonomy:

Kingdom	**:**	Plantae
Division	**:**	Magnoliophyta
Class	**:**	Magnolipsida
Order	**:**	Rosales
Family	**:**	Moraceae
Genus	**:**	Ficus
Species	**:**	*F. carica*

Ficus is a pan-tropical genus of trees, shrubs and vines occupying a wide variety of ecological niches; most are evergreen, but some deciduous species are endemic to areas outside of the tropics and to higher elevations [18]. Fig species are characterized by their unique inflorescence and distinctive pollination syndrome, which utilizes wasp species belonging to the Agaonidae family for pollination.

2.2. Macroscopic character

Ficus constituted one of the largest genera of medicinal plants with about 750 species of woody plants, trees, and shrubs primarily occurring in subtropical and tropical regions throughout the world. The genus are remarkable for the large variation in the habits of its species [19]. In India, the most important species of Ficus are *F. bengalensis*, *F. carica, Ficus racemosa* and *F. elastica. Ficus carica* is

commonly referred as Fig. Various parts of the plant like bark, leaves, tender shoots, fruits, seeds, and latex are medicinally important. The fig is a very nourishing food and used in industrial products. It is rich in vitamins, mineral elements, water, and fats. Figs are one of the highest plant sources of calcium and fiber. According to USDA data for the Mission variety, dried figs are richest in fiber, copper, manganese, magnesium, potassium, calcium, and vitamin K, relative to human needs. They have smaller amounts of many other nutrients. Figs have a laxative effect and contain many antioxidants. They are good source of flavonoids and polyphenols [20] and some bioactive compounds such as arabinose, β-amyrins, β-carotines, glycosides, sitosterol and xanthotoxol [21-23]. The dried figs produced a significant increase in plasma antioxidant capacity and also used in various disorders such as gastrointestinal respiratory, inflammatory, cardiovascular disorders, ulcerative diseases, and cancers [24-27].

In traditional medicine the roots are used in treatment of leucoderma and ringworms and its fruits which are sweet, have antipyretic, purgative, aphrodisiac properties and have shown to be useful in inflammations and paralysis [28, 29]. *F. carica* has been reported to include antioxidant, antiviral, antibacterial, hypoglycemic, cancer suppressive, hypotriglyceridaemic, and anthelmintic effects [30-32]. This study was aimed to present an overview of traditional, phytochemical and pharmacological investigations of bioactive compounds present in this plant.

Figure 4: Anjir plant

2.3. Medicinal Uses of Fig

1. Grind four fig leaves along with sugar candy. Regularly take this mixture along with a glass of water two times in a day. This fig home remedy is useful in the natural treatment of liver cirrhosis.
2. Consuming one teaspoon of fig seeds along with a teaspoon of honey daily to control diabetes**.** Follow this therapy for about a week.
3. Fig benefits in removing kidney stones by having a cup of fresh fig juice for a few days. In addition, you can take a solution of six figs boiled in a cup of water. Follow this fig remedy for about a month [33]
4. Take a cup of buttermilk and add a teaspoon of powdered fig bark in it. Drink this solution daily for a few days to get rid of diarrhea.
5. When dealing with excessive menstruation, apply freshly ground ten fig leaf buds on the lower abdomen. Leave it for a couple of hours and repeat the procedure a few times.
6. Soak a couple of figs in milk overnight and consume them next morning to increase sexual vitality.
7. Those suffering from anemia can eat 2-3 figs soaked in a cup of water overnight. Consume these figs along with milk in the morning for a month.
8. Take one and a half liters of water and add 50 g each of dried figs**,** barley and raisins in it. Boil the solution. Simmer and keep it covered for about 15 minutes. Next, add 15 g of chopped licorice root in it.
9. Cover and leave it to stand overnight. Finally, stain the liquid and take one teaspoon of this solution. This is an effective figs home remedy for constipation in children.
10. Applying the milky white juice of fig leaves on the affect areas cures corns and calluses. It is useful in treating insect bites, too.
11. Chewing a couple of tender fig leaves and then rinsing the mouth with warm water reduces bad breath and mouth ulcers [34].

2.4. Review of Literature

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- Vikas. V patil, et al, gave a over view on *Ficus carica* Linn, Research Jouralof Medicial Plants [37].
- Baby Joseph and S.Justin Raj. Pharmacognostic and phytochemical properties of *Ficus carica* Linn [38].
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- Mohamed Bnouham et al, has reported the nature of Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research [41].
- P. A. Akah, et al reported the Antidiabetic activity of aqueous and methanol extract and fractions of *Gongronema latifolium* (Asclepidaceae) leaves in Alloxan Diabetic Rats [42].
- Mishra Shanti Bhusan et al reported the analytical review of plants for anti-diabetic activity with their phytoconstituent & mechanism of action [43].
- Kanika pandit, et al, has evaluate the anti hyperlipidemic activity of *Ficus hispida* Linn leaves. International journal of pharmacy and pharmaceutical sciencesvol-3, iss-5, 188-191, 2011 [44].

AIM AND OBJECTIVES OF STUDY

AIM

To evaluate Antidiabetic and Antioxidant Effects of Ethanolic Extract of *Ficus carica* Leaves In Alloxan Induced Diabetic Rat.

OBJECTIVES OF THE STUDY

The objectives of the present study is to

- Identify the phytochemical constituents of the medicinal plant *Ficus carica*
- To determine the acute toxicity profile of plant *Ficus carica*.
- To authenticate antidiabetic effect of the medicinal plant through standard evaluation techniques.
- To evaluate antioxidant property of *Ficus carica*

3. PLAN OF WORK

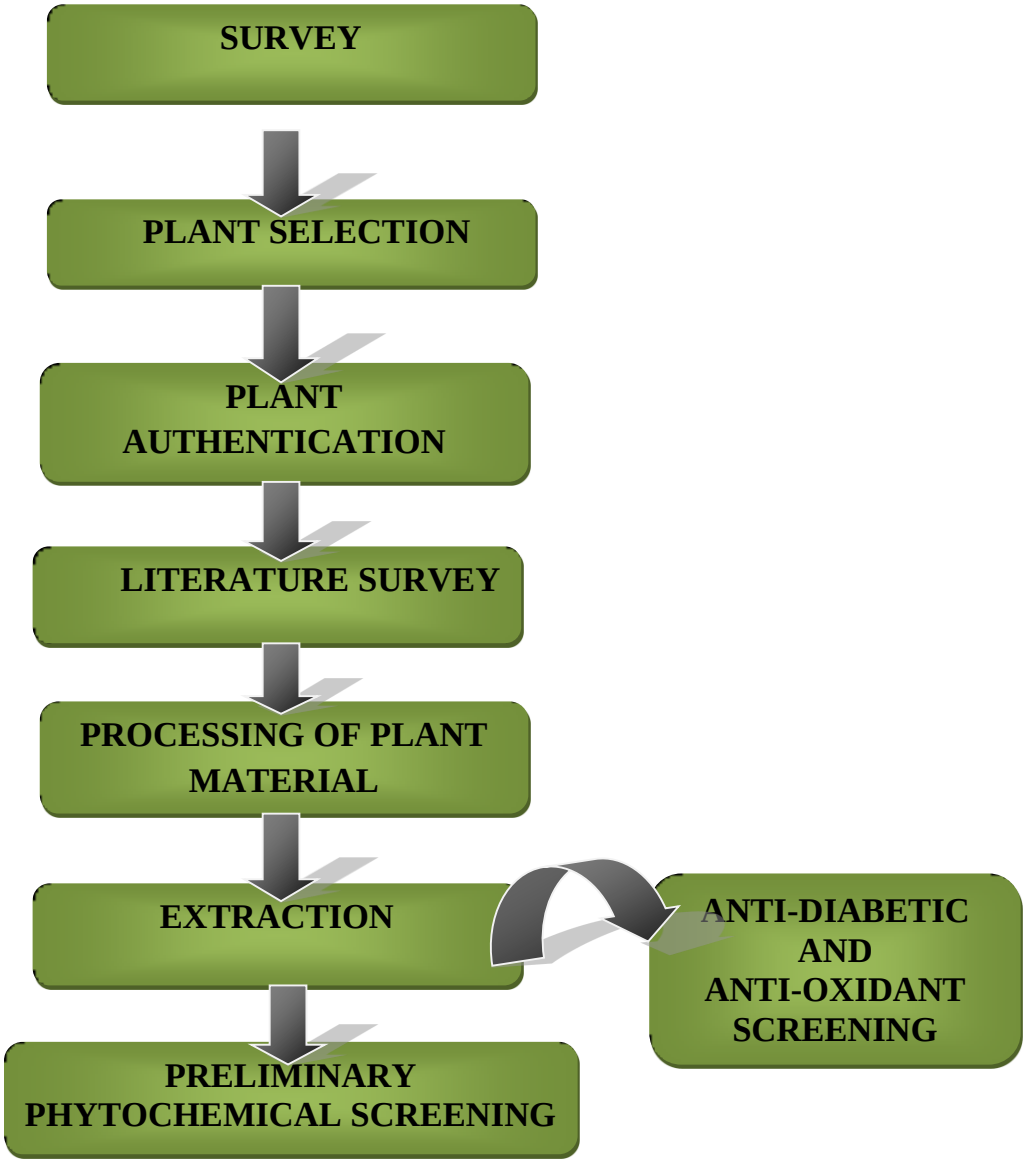

Figure 5: Plan of work

4. MATERIALS AND METHODS

4.1. Collection and authentication of *Anjir* leave

Leaves of *Anjir* leaves were collected in June 2013 from Sri Venkateswara University, Tirupati, India. The leaves were verified and Authenticated by **Dr. K. Madhava Chetty,** Assistant Professor, Department of Botany, Tirupathi. The leaves were dried in shade and made into coarse powder.

4.2. Preparation of ethanolic extracts of *Anjir* leaves

The leaves were separated from the plant and it was washed with absolute ethanol to avoid the microbial growth, the leaves were dried at open air under the shade, cut in to small pieces and powdered mechanically, then 50 gm of powder *Anjir* leaves was extracted with 250 ml ethanol in a soxhlet apparatus for 72 hrs. The extract obtained was concentrated by recovery of ethanol. The concentrated product was used as ethanolic extract of leaves of *Anjir*.

4.3. Phytochemical investigation on Anjir leaves [45, 46, 47]

4.3.1. Test for saponins

Foam test

Take 2 ml of drug solution in a test tube, add small amount of water to it, shake well, stable froth (foam) is formed.

4.3.2. Test for tannins

a. Ferric chloride test

A small amount of test solution treat with ferric chloride solution, blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

b. Phenazone test

To the 5 ml of aqueous extract add 0.5 gm of sodium acid phosphate. Then warm it and filter. To the filtrate, add 2% Phenazone solution, precipitate is formed which is often colured.

c. Gelatin test

To the test solution add 1% gelatin solution containing 10% sodium chloride. Precipitate is formed.

4.3.3. Test for amino acids

a. Millon's test

To the test solution, add 2 ml of millons reagent, white precipitate indicates presence of amino acid.

b. Ninhydrin test

To the test solution, add ninhydrin solution, boil, violet color indicates presence of amino acid.

4.3.4. Test for proteins

a. Warming test

The test solution take in a test tube and heat in boiling water bath, proteins get coagulated.

b. Test with trichloro acetic acid

To the test solution add trichloro acetic acid, precipitate is formed.

c. Biuret test

To the test solution (2 ml) add Biuret reagent (2 ml), violet color indicates presence of proteins.

d. Hydrolysis test

Hydrolyze the test solution with hydrochloric acid or sulphuric acid. Then carry out the ninhydrin test for amino acid.

e. Xanthoproteic test

To the 5ml of test solution, add 1ml of concentrated nitric acid and boil, yellow precipitate is formed. After cooling it, add 40 %sodium hydroxide solution, orange color is formed.

4.3.5. Glycosides

a. Keller kiliani test

The test consists of boiling about 1 gm finely powered sample with 10 ml 70% alcohol for 2 to 3 minutes. The extract is filtered. To the filtrate is added, 5 ml water and 0.5 ml strong solution of lead acetate. Shake well and separate the filtrate. The clear filtrate is treated with equal volume of chloroform and evaporated to yield the extractive. The extractive is dissolved in glacial acetic acid and after cooling, 2 drops ferric chloride solution is added to it. These contents are transferred to a test tube containing 2 ml concentrated sulphuric acid. A reddish brown layer acquiring bluish-green color after standing is observed.

b. Legal test

The extract is dissolved in pyridine, sodium nitroprusside solution is added to it and made alkaline-pink or red color is produced.

c. Baljet test

To the section of sample, sodium picrate solution is added. It shows yellow to orange color.

4.3.6. Test for cardiac glycosides

a. Keddes test

Extract the drug with chloroform, evaporate to dryness. Add one drop of 90% alcohol and 2 drops of 2% 3, 5-Di nitro benzoic acid in 90% alcohol. Make alkaline with 20% sodium hydroxide solution, purple color is produced. The color reaction with 3, 5-Di nitro benzoic acid depends on the presence of alpha, beta unsaturated lactones in the aglycone.

b. Keller-killiani test (Test for deoxy sugars)

Extract the drug with chloroform and evaporate it to dryness. Add 0.4 ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5 ml of concentrated sulphuric acid by the side of the test tube. Acetic acid layer shows blue color.

c. Raymonds test

Treat the test solution with hot methanolic alkali, violet color is produced.

d. Legals test

Treat the test solution with pyridine and alkaline sodium nitroprusside solution, blood red color appears.

e. Baljet test

Treat the test solution with picric acid or sodium picrate, orange color is formed.

4.3.7. Test for alkaloids

The qualitative chemical tests used for detection of alkaloids are dependent on the characters of alkaloids to give precipitates as salts of organic acids

or with compounds of heavy metals, like mercury, gold, platinum, etc. The different reagents used are Mayer's reagent (potassium mercuric iodide solution) giving cream colored precipitate. Dragendorff's reagent (potassium bismuth iodide solution) giving reddish brown precipitate. Wagner's reagent (iodine-potassium iodide solution) yielding reddish brown precipitate. Some alkaloids also give yellow coloured precipitates with picric acid called as Hagner's reagent and picrolonic acid. Individual alkaloid gives color or precipitate with certain specific reagent.

a. Dragendorff's test

To 2-3 ml filtrate, add few drops of Dragendorff's reagent. Orange brown precipitate is formed.

b. Mayer's test

2-3 ml filtrate with few drops of Mayer's reagent gives precipitate.

c. Hager's test

2-3 ml filtrate, add few drops of Hager's reagent gives yellow precipitate.

d. Wagner's test

2-3 ml filtrate with few drops of Wagner's reagent gives reddish brown precipitate.

4.3.8. Test for carbohydrates

a. Molisch's test

The test is positive with soluble, as well as, insoluble carbohydrates. It consists of treating the compounds with alpha napthol and concentrated sulphuric acid which gives purple color ring at the junctions of two layer.

b. Reduction of Fehling's solution

To the solution of sample, equal quantity of Fehling's solutions A and B is added. After heating, brick red precipitate was obtained.

4.3.9. Test for flavonoids

a. Shinoda test

To dry powder or extract, add 5 ml 95% ethanol, few drops concentrated HCl and 0.5 gm magnesium turnings. Pink color was observed.

To small quantity of extract, add lead acetate solution. Yellow colored precipitate was formed.

4.4. Acute toxicity study as per OECD guideline 425

In the assessment and evaluation of the toxic characters of the substance, determination of acute oral toxicity is usually an initial step. It provides information of health hazards likely to arise from a short-term exposure by the oral route. Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24h. Data from an acute study may serve as a basis for classification and labeling. LD (medium lethal 50 doses), oral, is a statistically derived single dose of a substance that can be expected to cause death in 50% of animals when administered by the oral route. The LD_{50} value expressed in terms of test substance per unit weight of test animal (mg/kg). It is initial step in establishing a dosage regimen in sub chronic and other studies and may provide initial information on the mode of toxic action of a substance.

The concept of the up and down (UDP, stair case method) was first designed by Dixon and Mood. In this method animals of a single sex, usually females, with the first animal receiving a dose just below the best estimate of the LD_{50}. Depending on the outcome for the previous animal, the dose for the next is increased or decreased, usually by the factor of 3.2. This sequence continues until

there is a reversal of the initial outcome (i.e., the point where an increasing dose results in death rather than survival or decreasing dose result in survival rather than death) then, additional animals are dosed following the up-down principle until a stopping criterion is met. If there is no reversal before reaching the selected upper (2000 or 5000 mg/kg) limit dose, then a specific number of animals are dosed at the limit dose. The option to use an upper limit dose of 5000 mg/kg should be taken only when justified by a specific regulatory need.

Healthy Wistar rats weighing between 180-220 g were used to carry out acute oral toxicity studies by the 'staircase' method. All successive extracts of *Anjir* leaves in 0.5% tween 80 was administered orally by gavages in graduated dose to several groups of experimental animals, one dose being used per group. Subsequently, observations of effects were made at 0,1,2,4 and 24 h for any mortality [48]. Ethical clearance for handling the animals is obtained from the Institutional animal ethical committee prior to the beginning of the project work from Institutional Animal Ethics Committee (769/2010/CPCSEA) approved the study protocol.

4.5. Experimental Designs

4.5.1. Anti-diabetic and Anti- oxidant activity of *Anjir* leaves (Alloxan-induced diabetic model)

Alloxan monohydrate was first weighed individually for each animal according to their weight and then solubilized with 0.2 ml saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg body weight. intraperitonially. After 1 h of alloxan administration, the animals were given feed *ad libitum*, and 5% dextrose solution was also given in a feeding bottle for a day to overcome the early hypoglycemic phase. The animals were kept under observation and after 48 h blood glucose was measured by glucometer. The diabetic rats (glucose level >300 mg/dl) were separated and divided into five different groups for experimental study, with each group containing six animals [49]

Group I	Normal Control (Saline)
Group II	Diabetic control (Alloxan induced)
Group III	Diabetic (Alloxan induced) + Glibenclamide (5 mg/kg)
Group IV	Diabetic(Alloxan induced) + *Anjir* leaf Extract 200 mg/kg
Group V	Diabetic (Alloxan induced) + *Anjir* leaf Extract 400 mg/kg

For all the animals the blood glucose level is measured on Day 0, 5, 10 and 15. The results of group IV and group V (Anjir leaf extract treated groups) is compared with group I, II (control groups) and group III (standard drug treated group).

Blood samples collected from all the animals are further subjected to tests for determining Lipid profile, Hepatic glycogen level and tissue concentration of LPO, SOD and CAT. The results of the Anjir leaf extract treated groups are compared that with the control and standard drug treated groups.

4.6. Statistical analysis

Statistical analysis of the results was done using the statistical functions of the Graphpad Prism 5.0 software. The results were expressed in terms of mean ± SD. The significance of difference between mean values for the various treatments were tested using one way analysis of variance test (ANOVA test) followed by Dunnett Multiple Comparisons Test and the p values less than 0.05 were considered significant [50].

5. RESULTS AND DISCUSSION

5.1. Collection and authentication of *Anjir* leaves

Leaves of *Anjir* Leaves was collected and authenticated. The collected leaves were dried in shade and made into coarse powder. The coarse powder of *Anjir* Leaves was used for further process.

5.2. Preparation of ethanolic extracts of *Anjir* leaves

The methanolic extract of *Anjir*Leaves was prepared according to the procedure discussed in materials and methods. The concentrated product was used in phytochemical and pharmacological screening.

5.3. Phytochemical investigation on *Anjir* leaves

Thepreliminary phytochemical screening like Saponins, Tannins, Amino acids, Proteins, Glycosides, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids was done with the methanolic extract of *Anjir* Leaves according to the procedure. In the above chemical test the methanolic extract of *Anjir* Leaves showed positive results for Saponins, Tannins, Amino acids, Proteins, Cardiac glycosides, Alkaloids, Carbohydrates and Flavonoids except glycosides. The results of preliminary test of methanolic extract of *Anjir* Leaveswere shown in Table 1.

Table 1: Phytochemical screening results of *Anjir* leaves

S. NO	PHYTOCONSTUTIENT	RESULT
1.	SAPONINS	+
2.	TANNINS	+
3.	AMINO ACIDS	+
4.	PROTEINS	+
5.	GLYCOSIDES	-
6.	CARDIAC GLYCOSIDES	+
7.	ALKALOIDS	+
8.	CARBOHYDRATES	+
9.	FLAVONOIDS	+

PRESENT = (+), ABSENT = (-)

5.4. Acute toxicity study as per OECD guideline 425

Acute toxicity test at 3000 mg/kg of leaf extracts of *Anjir* Leaves produced no mortality after 24 hours of observation. The median lethal dosage (LD_{50}) of the ethanolic leaves extract was greater than 3000 mg/kg body weight. The extract did not produce any grossly negative behavioral changes such as excitement, restlessness, respiratory distress, convulsions or coma. However, a reduction in body weights of rats was observed. The reduction in body weight may be due to reduced fluid and water intake, which may be secondary to feeling of fullness and loss of appetite after administration of the extract. Despite the above side effects, the very high value of the LD_{50} indicated that the extract of *Anjir* leaves is practically non-toxic.

5.5 Anti-diabetic and Anti- oxidant activity of *Anjir* leaves (Alloxan-induced diabetic model)

Phytochemical screening of all the extract of *Anjir* leavesshowed the presence of various chemical constituents, mainly tannins, saponins and flavonoids which may be responsible for its antidiabetic and anti-oxidant properties was shown in Table 1. The results obtained were comparable and satisfied the standard literature. To ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by alloxan-induced model. As expected, in the diabetic control, there was severe hyperglycemia when compared with the normal animals. When compared with the Diabetic control, the ethanolic extract of Anjir leaves as shown inTable 2 and Figure 5, lowered the elevated blood glucose levels. It was observed that the standard drug Glibenclamide lowered the blood glucose level significantly bringing it nearly back to normal, whereas ethanolic extract of *Anjir* leaves significantly decreased blood serum glucose in the diabetic rats on fifth, tenth, fifteenth and twentieths days compared with the diabetic control rat’s blood serum glucose levels.

In the present study, diabetic rats had lower body weights, high blood glucose level as compared to the normal rats, which confirmed the induction of diabetic by alloxan. In spite of the increased food consumption, loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics. The treatment with ethanolic extract of *Anjir* leaves improved the average body weights of rats which indicate control over polyphagia and muscle wasting resulted due to hyperglycemic condition.

Alloxan causes massive reduction in insulin release, through the destruction of β-cells of the Islets of Langerhans. In our study, we have observed a significant increase in the plasma insulin level when alloxan diabetic rats were treated with *Anjir* leaves. This could be due to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells of islets of Langerhans or its release from bound insulin. The significant and consistent antidiabetic effect of *Anjir* leaves in alloxan diabetic rats may also be due to enhanced glucose utilization by peripheral tissues.

Table 2: Anti Diabetic Activity of Ethanolic Extract of *Anjir* leaves on Alloxan Induced Diabetic Rats

Group No.	Treatment group	Day 0	Day 5	Day 10	Day 15	Day 20
I	Normal control	98.67±3.45	92.56±6.23	85.87±8.53	91.23±5.89	87.48±6.74
II	Diabetic control	375.39±13.67	398.45±16.72	407.51±13.34	436.67±15.49	452.74±18.39
III	Diabetic + Glibenclamide	368.73±10.45	304.92±14.39	250.79±14.27	233.44±11.25	208.25±11.59
IV	Diabetic + Extract 200 mg/kg	372.21±12.34	364.32±06.53	336.12±04.98	343.46±12.22	295.54±06.74
V	Diabetic + Extract 400 mg/kg	377.63±12.53	352.38±15.64	316.37±16.83	284.79±10.64	253.70±12.92

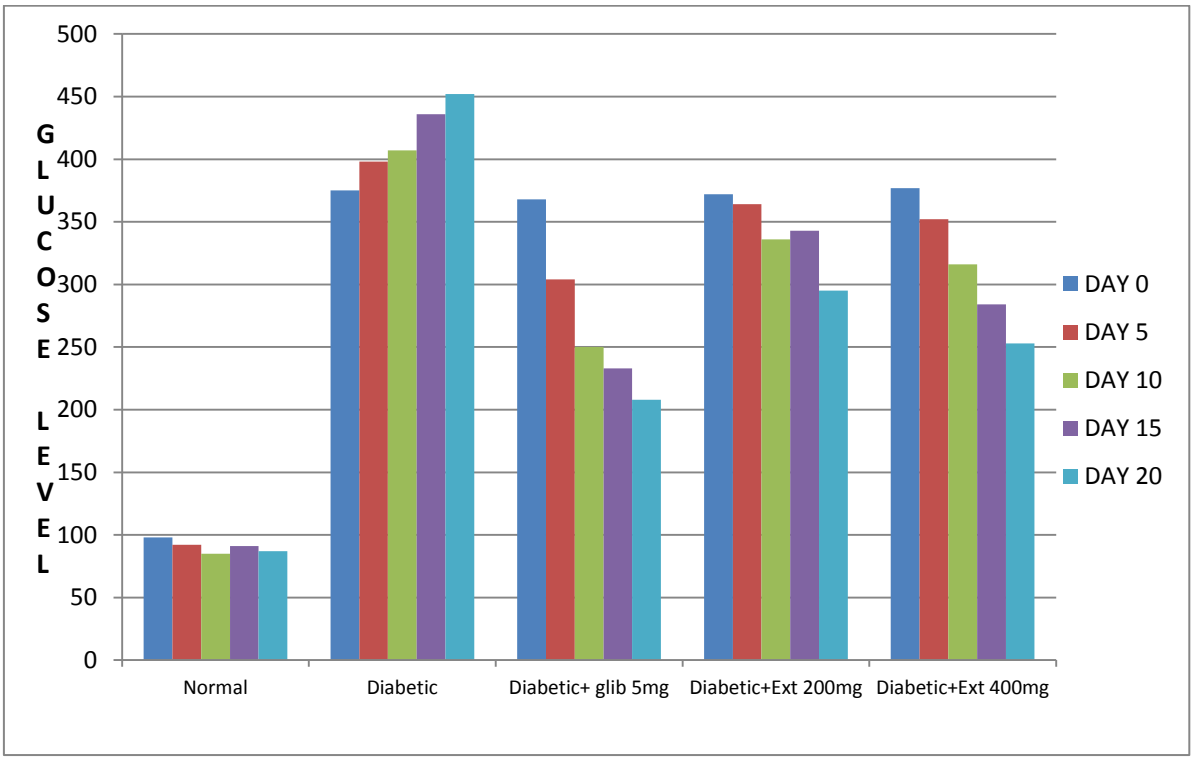

Figure 6: Anti Diabetic Activity of Ethanolic Extract of *Anjir* leaves

The serum lipid profile in Table 5 and Figure 8 treated with *Anjir* leaves extract returned to values nearing that of the control group. This showed that treatment with *Anjir* leaves significantly improved the lipid profile in diabetic animals and reduces the glycogen level in liver shown in Table 4 and Figure 7. Table 3and Figure 6 show the concentration lipid peroxidation and hydroperoxides in the liver of both control and experimental groups of rats. There was a significant elevation in tissue lipid peroxidation and hydroperoxides in diabetic rats. Administration of *Anjir* leaves to diabetic rats decreased the levels of tissue lipid peroxidation and hydroperoxides to normal levels. The concentration of tissues LPO, SOD and CATwere significantly decreased in diabetic rats when compared to the control group. Administrations of *Anjir* leaves extract to diabetic rats tend to bring the activities of these enzymes to near normal level.

Table 3: Antioxidant Activity of Ethanolic Extract of Anjir leaves on Alloxan Induced Diabetic Rats

Group No.	Treatment group	LPO	SOD	CAT
		No. of moles MDA formed/mg of protein	Amount of protein required for 50% inhibition	Moles of H2O2 decomposed/minute/mg of protein
I	Normal Control	2.86	7.27	93.64
II	Diabetic (Alloxan induced) control	4.23	3.89	49.37
III	Diabetic (Alloxan induced)+ Glibenclamide	3.12	6.53	81.35
IV	Diabetic (Alloxan induced) + Extract 200 mg/kg	4.16	4.20	55.26
V	Diabetic (Alloxan induced) + Extract 400 mg/kg	3.59	5.66	72.89

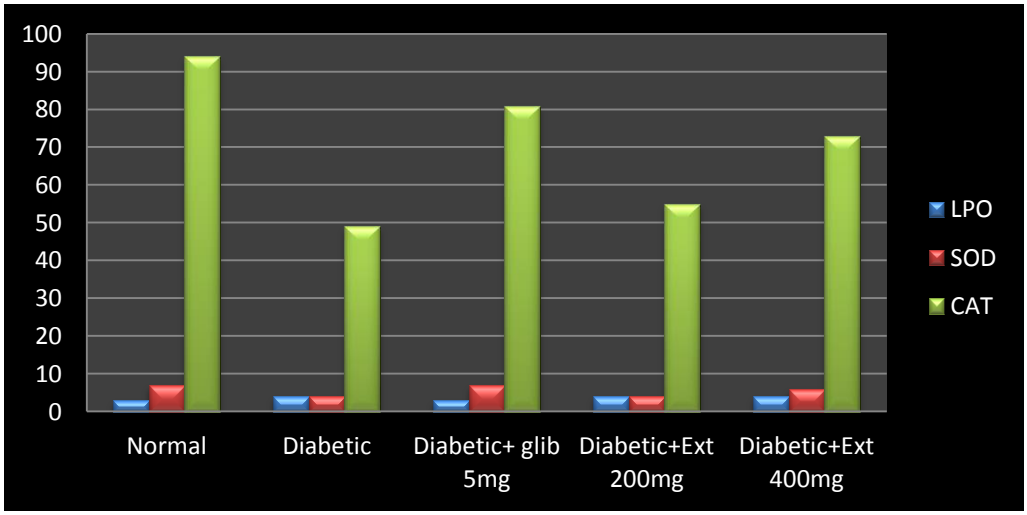

Figure 7:Antioxidant Activity of Ethanolic Extract of Anjir leaves

Table 4: Hepatic Glycogen Level of Ethanolic Extract of Anjir leaves on Alloxan Induced Diabetic Rats

Group No.	Treatment group	Liver glycogen (mg/g)
I	Normal Control	42
II	Diabetic (Alloxan induced) control	15
III	Diabetic (Alloxan induced) + glibenclamide	36
IV	Diabetic (Alloxan induced) + Extract 200 mg/kg	17
V	Diabetic (Alloxan induced) + Extract 400 mg/kg	23

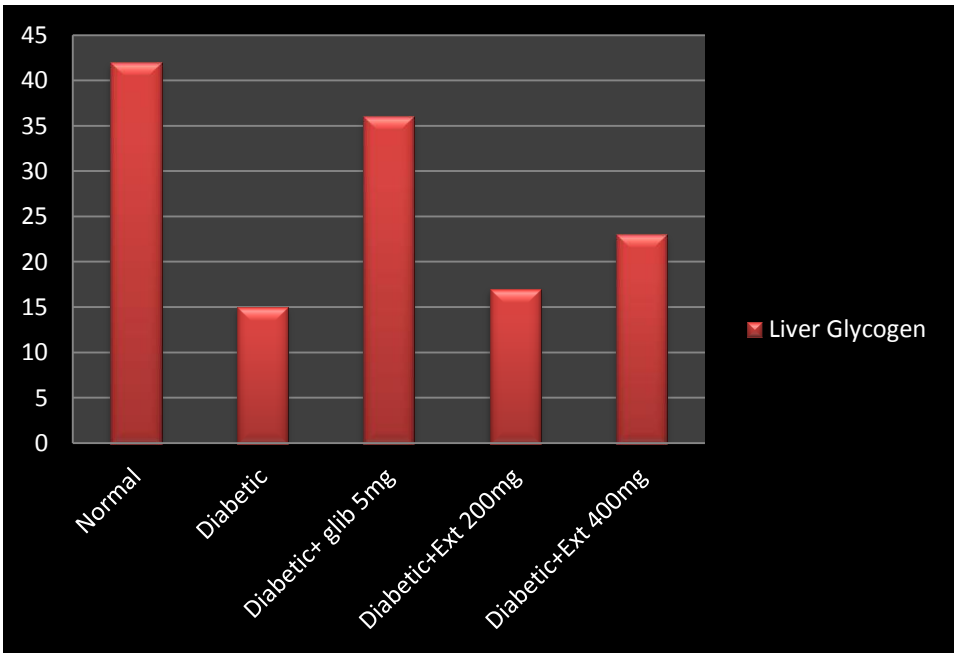

Figure 8:Hepatic Glycogen Level of Ethanolic Extract of Anjir leaves

Table 5: Serum Cholesterol Level of Ethanolic Extract of Anjir leaves on Alloxan Induced Diabetic Rats

Group No.	Treatment group	Serum cholesterol (mg/dl)
I	Normal Control	97
II	Diabetic (Alloxan induced) control	225
III	Diabetic (Alloxan induced) + glibenclamide	126
IV	Diabetic (Alloxan induced) + Extract 200 mg/kg	194
V	Diabetic (Alloxan induced) + Extract 400 mg/kg	152

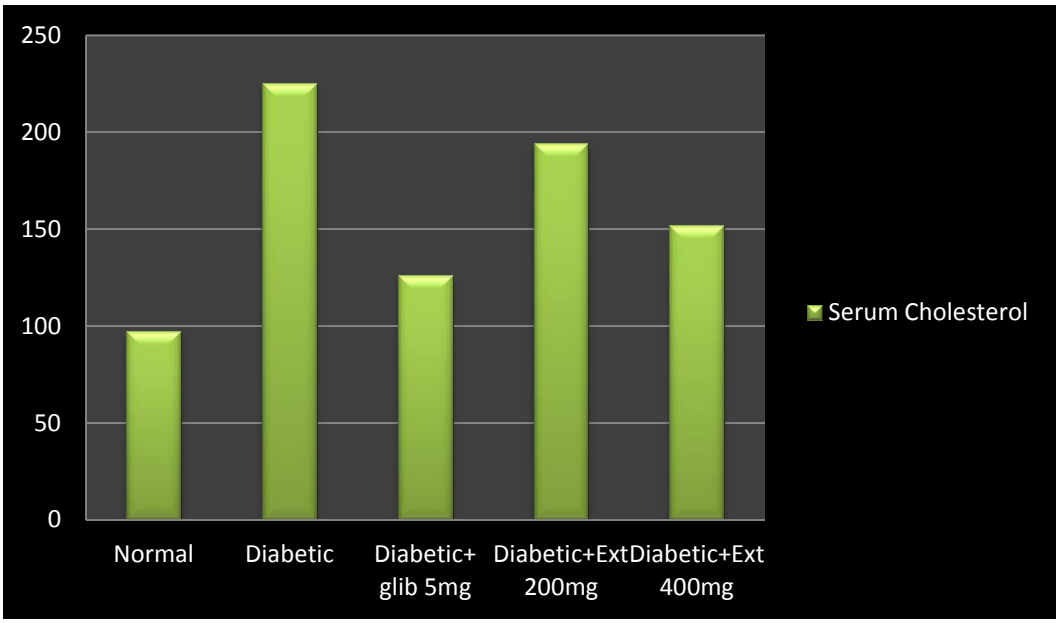

Figure 9:Serum Cholesterol Level of Ethanolic Extract of Anjir leaves

5.6. DISCUSSION

The currently-available drug regimens for management of diabetes mellitus have certain drawbacks and therefore, there is a need for safer and more effective antidiabetic drugs. This study was undertaken to assess the antidiabetic effect of *Anjir* leaves. In the present study, the oral treatment of *Anjir* leaves extract decreased the blood glucose levels in diabetic rats. It has been reported that using medicinal plant extract to treat alloxan-induced diabetic rats results in activation of *ß*-cells and insulinogenic effects. *Anjir* leaves may also have brought about hypoglycaemic action through stimulation of surviving *ß*-cells of islets of Langerhans to release more insulin. This was clearly evidenced by the increased levels of plasma insulin in diabetic rats treated with *Anjir* leaves. Since the percentage fall in plasma glucose levels was different in models with varying intensity of hyperglycaemia, it implies that the anti hyperglycaemic effect of that plant is dependent on the dosage of diabetogenic agent, which in turn leads to *ß*-cell destruction. A number of other plants have also been observed to exert hypoglycaemic activity through insulin release stimulatory effects. The concentrations of lipids, such as serum cholesterol were significantly higher in diabetic rats than in the control group. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, are responsible for the observed accumulation of lipids. The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Further, it has been reported that diabetic rats treated with Glibenclamide shows normalised lipid levels. Thus, the results indicate that *an Anjir leaf shows* insulin-like action by virtue of its lipid lowering levels. Oxidative stress has been shown to play a role in the causation of diabetes mellitus. Antioxidants have been shown to have a role in the alleviation of diabetes mellitus. In diabetes mellitus, oxygen free radicals (OFRs)are generated by stimulating H_2O_2. In our study, concentrations of lipid peroxides and hydroperoxides were increased in liver of diabetic rats, indicating an increase in the generation of free radicals. Increased lipid peroxidation in diabetes mellitus can be due to increased oxidative stress in the cell as a result of depletion of antioxidant scavenger systems. The present finding indicates significantly increased lipid

peroxidation of rats exposed to alloxan and its attenuation by *Anjir* leaves *treatment*. This suggests that the protective role of *Anjir* leaves extracts could be due to the antioxidative effect of flavonoids present in the leaf, which in turn act as strong superoxide radicals and singlet oxygen quenchers. Numerous studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes mellitus. In the current study, the LPO, SOD and CAT activities were significantly reduced in the liverof diabetic rats. These observations emphasize the critical importance of maintaining the antioxidant potential of the pancreatic ß-cell in order to ensure both its survival and insulin secretion capacity during times of increased oxidative stress. Thedecreased activities of SOD and CAT in theliverduring diabetes mellitus may be due to the production of reactive oxygen free-radical that can themselves reduce the activity of these enzymes.

6. SUMMARY AND CONCLUSION

1. Preliminary phytochemical analysis report data.

2. In this experimental model, the ethanolic extract *Anjir leaves* (200, 400 mg/kg) with reference standard Glibenclamide 5mg/kg significantly effective in abnormalities of enzyme profile in experimental rats.

3. The data that *Anjir leaves* extract is beneficial in controlling the blood glucose level, improves the lipid metabolism and prevents diabetic complications from lipid peroxidation and antioxidant systems in experimental diabetic rats.

4. This could be useful for prevention or early treatment of diabetic disorders.

5. We conclude that the *Anjir leaves* have potent anti-diabetic and anti-oxidant effects in alloxan induced diabetic rats.

6. The present investigation has also opened avenues for further research especially with reference to the development of potent formulation for diabetes mellitus from *Anjir leaves.*

7. Activity guided fractionation, formulation, and its evaluation is in progress and will be available in short period of time.

8. Data on the short and long term adverse effects of *Anjir leaves* ingestion needs to be collected.

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